METHODS
RNA extraction and real-time PCR

Total RNA was isolated from cells grown at an ALI. Three transwells from each experimental condition were harvested and pooled to isolate RNA by using the RNAqueous kit for total RNA purification from Ambion–Applied Biosystems. RNA concentration and integrity were determined by using the Agilent 2100 Bioanalyzer system and Agilent RNA 6000 Nano Chips (Agilent Technologies). RNA samples (1 μg) with an RNA integrity number of 8 or greater were reverse transcribed with Moloney murine leukemia virus reverse transcriptase with a combination of random hexamers and oligo-dTs by using the SuperScript VILO cDNA Synthesis Kit from Invitrogen. Samples were diluted up to a final volume of 100 μL (10 ng/μL). Semiquantitative real-time qPCR was performed by using the SensiMix II probe kit wit SYBR Green (Bioline). We designed specific primers and validated the optimal conditions for amplification of periostin (NM_006475.2; forward, 5′-ACAGCTCAGAG TCTTCGTATATCG-3′; reverse, 5′-CCGTTTCTCCCTTGCTTACTCC-3′) and GAPDH (NM_002046.3; forward, 5′-CATCCCTGCCTCTACTGGCG-3′; reverse, 5′-TAGACGGCAGGTCAGGTCCAC-3′). ADAM33 (NM_025 220) primers were purchased from SA Biosciences (Qiagen). A library of representative samples containing cDNAs were made, and serial dilutions of 1×, 0.1×, 0.01×, 0.001×, and 0.0001× were prepared for the validation of primers and to determine amplification efficiency. These dilutions were run in parallel with samples to adjust the efficiency in each set of samples analyzed. Samples of 15 μL were prepared by using 0.1 μmol/L of each of the forward and reverse primers and 1 μL of 1× cDNA.

Real-time qPCR reactions containing 1.5 μL of cDNA, 0.2 μmol/L primers, and the 2× SYBR Green Supermix were run in the Bio-Rad iQ thermal cycler (Bio-Rad Laboratories, Hercules, Calif) with programs specific for the respective gene. Reactions were run in triplicate, and genes of interest were normalized to the GAPDH housekeeping gene. All primers were designed in-house and optimized for efficiency, and the absence of primers-dimers was confirmed for primer specificity. Single amplicons of the appropriate base pair were confirmed by means of gel electrophoresis.

The relative expression of periostin and ADAM33 was standardized by using GAPDH as a nonregulated reference gene. The expression ratio was calculated by using the real-time PCR efficiency and the Δ cycle threshold (Ct; intersection between a threshold point and the amplification curve). We used the REST (Relative Expression Software Tool)–MCS software to compare gene expression.[E1] The software uses PCR efficiencies and the average crossing point deviations among subject groups (asthmatic, atopic nonasthmatic, and healthy groups). The expression ratios are tested for statistical significance by using a Pair Wise Fixed Re-allocation Randomisation Test and plotted by using SE estimation through the Taylor algorithm.[E2] All PCR analysis algorithms used to estimate relative gene expression use changes in Ct values. The relative expression ratio of a target gene is computed based on its real-time PCR efficiencies (E) and the difference (Δ) of 1 unknown sample (treatment) versus 1 control (ΔCt control − treatment). The Pfaffl algorithm is based on the following equation:

$$\text{Ratio} = E(\text{target})^{\Delta\text{Ct target (Mean control−Mean sample)}} / E(\text{reference})^{\Delta\text{Ct reference (Mean control−Mean sample)}}.$$

In addition to using this general algorithm, the REST software incorporates a Pair Wise Fixed Re-allocation Randomization Test to test for statistical significance and the Taylor algorithm to plot by SE estimation.

Our final PCR results were expressed as the fold increase on a $\log_2$ scale relative to median expression of genes in healthy control samples.

REFERENCES
E1. REST (Relative Expression Software Tool). Available at: http://rest.gene-quantification.info/. Accessed December 14, 2011.
E2. Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 2002;30:e36.
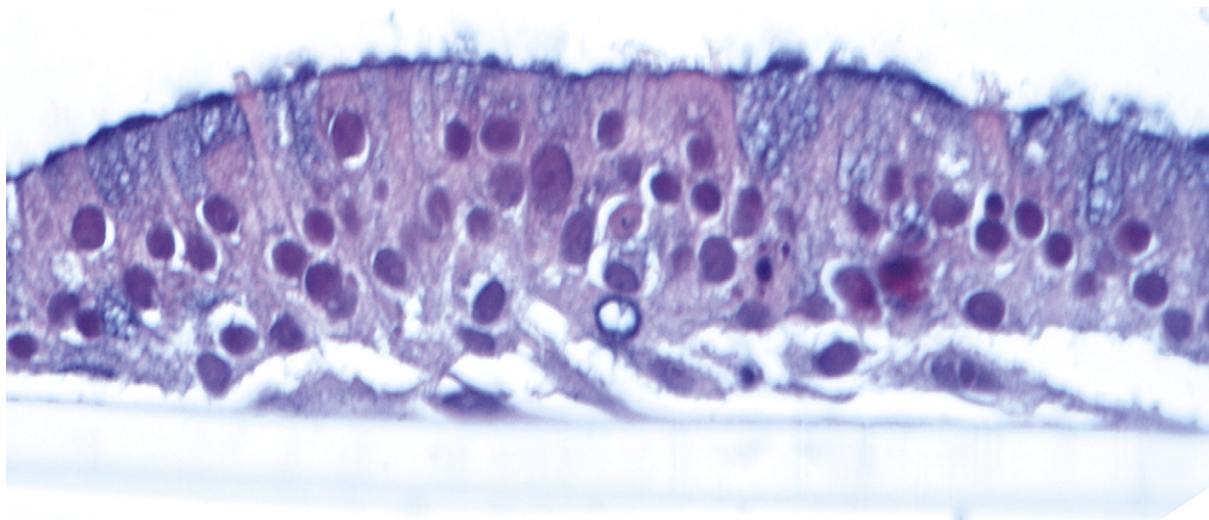
FIG E1. Hematoxylin and eosin–stained bronchial epithelial cells from an asthmatic patient differentiated at an ALI.
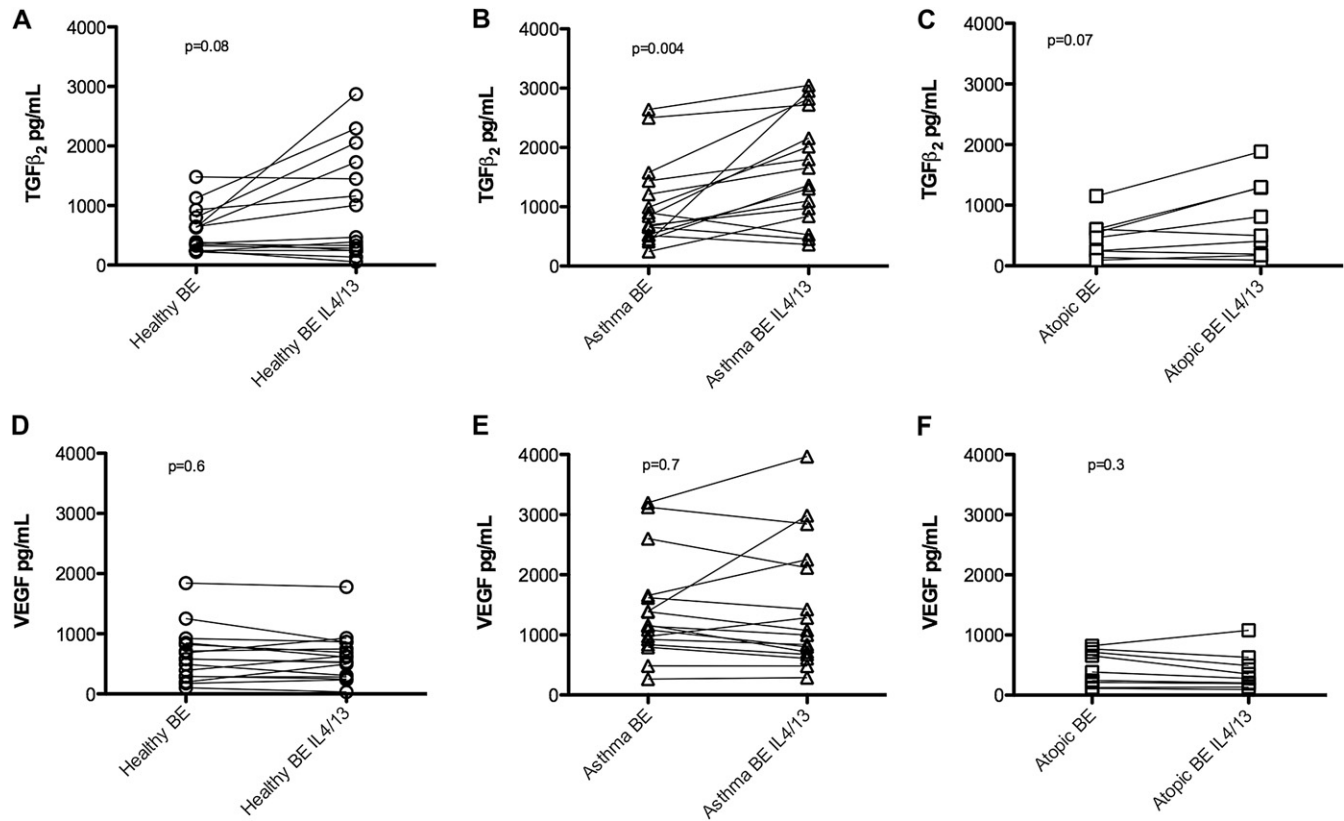
FIG E2. Change in TGF-β2 concentrations in ALI conditioned culture media from healthy bronchial **(A)**, asthmatic bronchial **(B)**, and atopic nonasthmatic bronchial **(C)** epithelial cells after stimulation with IL-4 and IL-13. Change in VEGF concentrations in ALI conditioned culture media from healthy bronchial **(D)**, asthmatic bronchial **(E)**, and atopic nonasthmatic bronchial **(F)** epithelial cells after stimulation with IL-4 and IL-13. Analyses were conducted with paired *t* tests or Wilcoxon matched-pairs test, as appropriate.

A

B
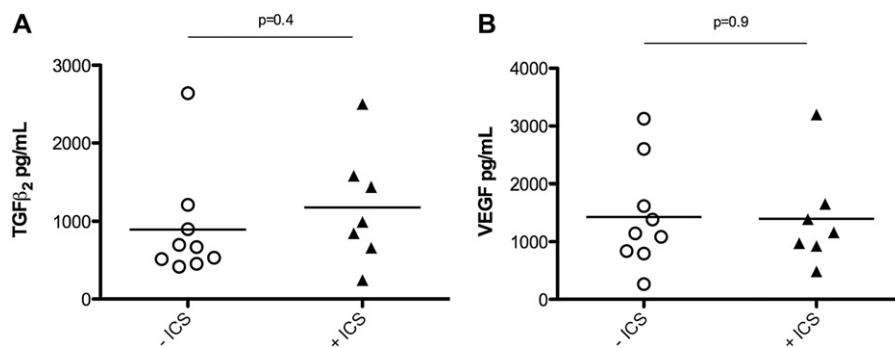
FIG E3. Production of TGF-β2 **(A)** and VEGF **(B)** in bronchial epithelial cell ALI cultures from asthmatic children who reported use of ICSs at the time of airway brushings *(+ICS)* was similar to production in cells from asthmatic children who were not using inhaled steroids *(−ICS)*.
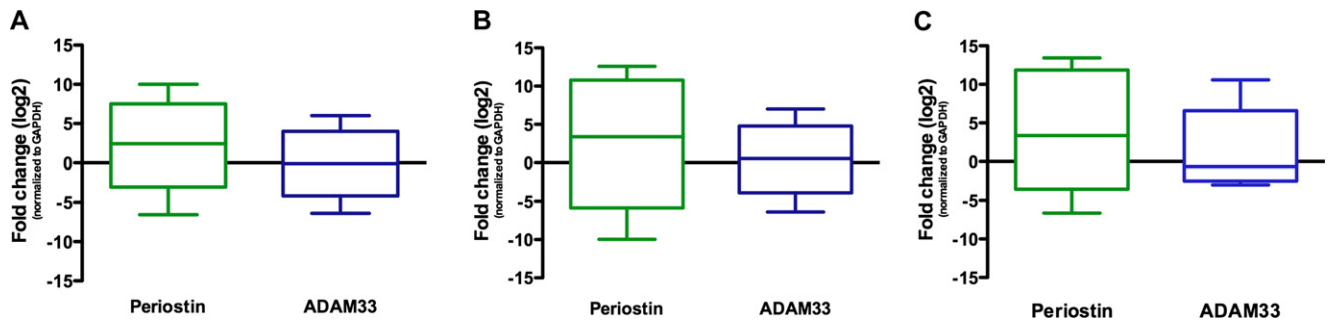
FIG E4. Periostin expression *(green box)* was 2.4-fold higher in bronchial epithelial cells from asthmatic pa-
tients stimulated with IL-4 and IL-13 compared with that seen in unstimulated cells from asthmatic patients
(**A**; *P* = .046). There was no significant change in periostin expression after IL-4 and IL-3 stimulation by bron-
chial epithelial cells from healthy subjects (**B**; *P* = .5) or atopic nonasthmatic patients (**C**; *P* = .4). ADAM33
expression *(blue box)* by AECs from asthmatic (Fig E4, *A*), healthy (Fig E4, *B*), or atopic nonasthmatic (Fig E4,
C) children did not change in response to IL-4 and IL-13 stimulation.
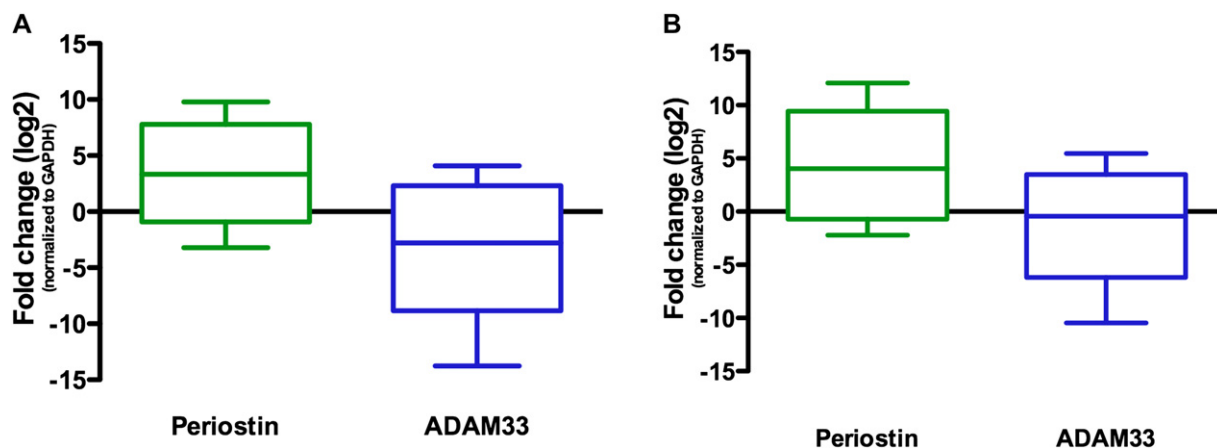
FIG E5. Periostin expression *(green box)* was 3.3-fold higher in asthmatic bronchial epithelial cells from children not using inhaled steroids *(−ICS)* than expression in healthy children (**A**; *P* = .003). Periostin expression in asthmatic children who were using inhaled steroids *(+ICS)* was 4-fold higher than expression in cells from healthy children (**B**; *P* = .001). ADAM33 *(blue box)* was not differentially expressed by either −ICS (Fig E5, *A*; *P* = .2) or +ICS (Fig E5, *B*; *P* = .9) asthmatic children in comparison with healthy children.